# Ascorbic acid and photosynthetic pigments in curly lettuce grown in a hydroponic

# system with saline solutions

Ácido ascórbico e pigmentos fotossintéticos na alface crespa cultivada em sistema hidropônico com soluções salinas

Ácido ascórbico y pigmentos fotosintéticos en lechuga crespa cultivada en sistema hidropónico con soluciones salinas

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### Abstract

The objective of this research was to determine the levels of chlorophyll and vitamin-C in the cultivars of lettuce (Elba, Cristina and Veneranda) in the hydroponic system with nutrient solutions with different levels of salinity. The experiment was conducted at the State University of Paraíba,UEPB Campus-II, rural area of the city of Lagoa Seca-PB, using the Laminar Flow of Nutrients-NFT technique in a protected environment. The experimental design was in randomized blocks, in a subdivided plot scheme, the plots consisting of seven nutrient solutions prepared from the Furlani methodology so that solution S<sub>1</sub> presented Electric conductivity-EC (1.9 dS m<sup>-1</sup>) and the other solutions, EC values: S<sub>2</sub>-2.4; S<sub>3</sub>-2.9; S<sub>4</sub>-3.4; S<sub>5</sub>-3.9; S<sub>6</sub>-4.4 and S<sub>7</sub>-4,9 dS m<sup>-1</sup>, with three repetitions. The subplots composed of the cultivars Elba, Cristina and Veneranda. Variables analyzed: levels of total chlorophyll (*Chl a* and *b*), carotenoids and vitamin-C. The nutritional solutions showed linear behavior for all treatments, not differing statically from each other. The highest levels were found in the cultivar Veneranda whose values 35.97 and 52.06 mg g<sup>-1</sup> respectively. The treatment Elba/S<sub>3</sub>=3.4 dS m<sup>-1</sup> provided the highest average carotenoid content of 57 mg g<sup>-1</sup>, yet the cultivar Elba showed the highest levels (*Chl a*) with nutrient solutions S<sub>2</sub>=2.4 and S<sub>3</sub>=3.9 dS m<sup>-1</sup>. The cultivar Veneranda promoted the highest levels for (*Chl b* and total). The highest levels of vitamin-C were found in the cultivars Cristina and Veneranda. Keywords: *Lactuca sativa* L.; Hydroponics; Salinity; SPAD-502; Chlorophyll.

### Resumo

O objetivo desta pesquisa foi determinar os teores de clorofila e vitamina-C nas cultivares da alface (Elba, Cristina e Veneranda) cultivadas em sistema hidropônico com soluções nutritivas apresentando diferentes níveis de salinidade. O experimento foi conduzido na Universidade Estadual da Paraíba,UEPB Campus-II, zona rural da cidade de Lagoa Seca-PB, via técnica Fluxo Laminar de Nutrientes-NFT em ambiente protegido. O delineamento experimental foi em

blocos casualizados, em esquema de parcelas subdivididas, as parcelas constituídas por sete soluções nutritivas preparadas a partir da metodologia de Furlani de modo que a solução S<sub>1</sub> apresentou Condutividade Elétrica-CE (1,9 dS m<sup>-1</sup>) e as demais soluções os seguintes valores de CE: S<sub>2</sub>-2,4; S<sub>3</sub>-2,9; S<sub>4</sub>-3,4; S5-3,9; S<sub>6</sub>-4,4 e S<sub>7</sub>-4,9 dS m<sup>-1</sup>, com três repetições. As subparcelas foram compostas pelas cultivares Elba, Cristina e Veneranda. As variáveis analisadas foram: teores de clorofila (*Chl a e b*) total, carotenoides e vitamina-C. As soluções nutritivas apresentaram comportamento linear para todos os tratamentos não deferindo estaticamente entre si. O maior valor para clorofila total foi obtido na cultivar Veneranda. Para as variáveis (*Chl b e total*) os maiores teores encontrados foi na cultivar Veneranda, cujos valores foram de 35,97 e 52,06 mg/g<sup>-1</sup> respectivamente. O tratamento Elba/S<sub>3</sub>=3,4 dS m<sup>-1</sup> proporcionou o maior teor de carotenoides média de 57 mg/g<sup>-1</sup>, ainda a cultivar Elba apresentou os maiores teores para (*Chl b e total*). Os maiores teores de vitamina-C foram encontrados nas cultivares Cristina e Veneranda. **Palavras-chave:** *Lactuca sativa* L.; Hidroponia; Salinidade; SPAD-502; Clorofila.

#### Resumen

El objetivo de esta investigación fue determinar los niveles de clorofila y vitamina-C en cultivares da lechuga (Elba, Cristina y Veneranda) cultivados en sistema hidropónico con soluciones nutritivas con diferentes niveles de salinidad. El experimento se realizó en la Universidad Estatal de Paraíba, UEPB Campus-II, área rural de la ciudad de Lagoa Seca-PB, la técnica de Flujo Laminar de Nutrientes-NFT en ambiente protegido. El diseño experimental en bloques aleatorios, esquema de parcelas subdivididas, las parcelas consistieron soluciones nutritivas preparadas a partir la metodología Furlani, de manera que la solución  $S_1$  presentó Conductividad eléctrica-CE (1.9 dS m<sup>-1</sup>) y las otras soluciones con CE: S<sub>2</sub>-2.4; S<sub>3</sub>-2.9; S<sub>4</sub>-3.4; S<sub>5</sub>-3.9; S<sub>6</sub>-4.4 y S<sub>7</sub>-4.9 dS m<sup>-1</sup>, con tres repeticiones, las subparcelas compuestas los cultivares Elba, Cristina y Veneranda. Variables analizadas fueron: niveles de clorofila total, Chl a, b, carotenoides y vitamina-C. Las soluciones nutricionales mostraron un comportamiento lineal para todos los tratamientos, no difiriendo estáticamente entre sí. El mayor valor de clorofila total se obtuvo en el cultivar Veneranda. Para las variables (Chl b y total) los niveles más altos se encontraron en el cultivar Veneranda, cuyos valores fueron 35.97 y 52.06 mg/g<sup>-1</sup> respectivamente. El tratamiento Elba/S<sub>3</sub>=3.4 dS m<sup>-1</sup> proporcionó el contenido promedio de carotenoides más alto de 57 mg/g<sup>-1</sup>, sin embargo, el cultivar Elba mostró los niveles más altos (*Chl a*) con soluciones nutritivas S<sub>2</sub>=2.4 y S<sub>3</sub>=3.9 dS m<sup>-1</sup>. El cultivar Veneranda promovió los niveles más altos para (*Chl b y total*). Los niveles más altos de vitamina-C se encontraron en los cultivares Cristina y Veneranda. Palabras clave: Lactuca sativa L.; Hidroponia; Salinidad; SPAD-502; Clorofila.

# **1. Introduction**

Chlorophyll can participate in energy transfer, where an excited chlorophyll molecule transfers its energy to another molecule (Taiz et al., 2017). One of the most important plant components are pigments, which are organic compounds capable of absorbing electromagnetic radiation in a range of 400 to 700 nm (Kluge et al., 2015).

The photosynthetic pigments present in vegetables vary according to species. Chlorophyll a (*Chl a*) is present in all organisms that perform photosynthesis. Photosynthetic bacteria are devoid of *Chl a* and have bacterioclorophyll in their place as a photosynthetic pigment. *Chl a* is the pigment used to perform photochemistry, while the other pigments assist in absorbing light and transferring radiant energy to the reaction centers, which is why they are called accessory pigments (Taiz & Zieger, 2013).

Vitamin C has antioxidant action where these chemical compounds can prevent or decrease the oxidative damage of lipids, proteins and nucleic acids caused by reactive oxygen species, which include free radicals, that is, antioxidants that have the ability to react with radicals free and thus restrict the harmful effects to the organism (Couto & Canniatti-Brazaca, 2010).

Salinity is a serious problem for agriculture, as it limits the growth and development of sensitive plants. In recent years, several studies have demonstrated the potential of using saline water in the production of vegetables in hydroponic cultivation (Santos et al., 2012).

Lettuce (Lactuca sativa L.) is a herbaceous plant, originating from a temperate climate, belonging to the Asteracea family and the subfamily Cichoriaceae, one of the most popular and consumed vegetables in the world and in Brazil. Lettuce stands out in the national scenario of hydroponic crops, being responsible for approximately 80% of the Brazilian agricultural production in this system (Alves et al., 2011).

The use of hydroponics has emerged as an alternative to problems such as the low availability of soils suitable for agriculture, increasing the efficiency of water, production, and improve food quality (Souza Neta et al., 2013).

The quality of the water used in the preparation of the nutrient solution is a fundamental factor to obtain high productivity and product quality, whether in cultivation using the NFT system (Gondim et al., 2010; Paulus et al., 2010) or in substrate cultivation (Dias et al., 2011; Santos et al., 2012). The nutritive solution for hydroponic cultivation is of fundamental importance, as the growth and development of the crop will depend on an adequate formulation (Oliveira et al., 2014). Oscillations in electrical conductivity (EC) are related to the consumption of water and nutrients by plants and the evaporation of nutrient solutions that occurred during the conduct of the experiment (Monteiro Filho et al., 2017). Silva et al. (2015) studied two levels of nutrient solution availability (2.75 and 5.50 L per pack of 24 plants) and four levels of water electrical conductivity (EC): 0.43 (control); 3.09; 6.87 and 8.53 dS m.

The objective of this research was to determine the levels of chlorophyll and vitamin C in the cultivars of curly lettuce (Elba, Cristina and Veneranda) grown in a hydroponic system with nutrient solutions with different levels of salinity.

## 2. Methodology

The experiment was conducted at the Center for Agricultural and Environmental Sciences-CCAA of the State University of Paraíba-UEPB, located in the rural area of the city of Lagoa Seca-PB, with the following geographical coordinates: (7°10′15″S, 35°51′14″W), according to the Köppen-Geige climate classification (Brazil, 1971). The municipality's climate is characterized as humid tropical, with an average annual temperature around 22 °C, the minimum being 18 °C and the maximum of 33 °C. The hydroponic system used was the Laminar Flow of Nutrients-NFT installed in a greenhouse. While conducting the experiment, the values of maximum, minimum, average temperature were recorded daily. The temperatures were recorded at 8:00 am, whose data are shown in Figure 1.





The design of the experiment was in randomized blocks, in a split-plot scheme, with the parcels constituted by seven nutritive solutions all prepared using the methodology of Furlani (1995) so that the  $S_1$  solution presented a CE of 1.9 dS m<sup>-1</sup> and solutions  $S_2$ ,  $S_3$ ,  $S_4$ ,  $S_5$ ,  $S_6$  and  $S_7$  the following EC values: 2.4; 2.9; 3.4; 3.9; 4.4 and 4.9 dS m<sup>-1</sup>, respectively. With the exception of solution  $S_1$ , the other solutions had their EC values increased by the addition of sodium chloride (NaCl). All

Source: Data from the present research.

nutrient solutions were prepared with rainwater with EC 0.156 dS  $m^{-1}$  in order to obtain a volume of 200 liters (L) of stock solution. The quantities of mineral fertilizers and the chemical composition of the mineral nutrient solution are shown in Tables 1 and 2.

Solution			
Salts	Furlani		
g 1000 L <sup>-1</sup> of water			
Ca $(NO_3)_2$ ;6H <sub>2</sub> O - Calcium nitrate	1000		
MAP - onoammonium phosphate	150		
DAP - Diamonium phosphate	-		
H <sub>2</sub> PO <sub>4</sub> - Phosphoric acid	-		
KH <sub>2</sub> PO <sub>4</sub> - Monopotassium phosphate	-		
KCl - Potassium chlorid	150		
KNO3 - Potassium nitrat	600		
MgSO <sub>4</sub> .7H <sub>2</sub> O- Magnesium sulfate	250		
MnCl.H <sub>2</sub> -O Manganese chloride	2.34		
Mn SO <sub>4</sub> .H <sub>2</sub> O - Manganese sulphate	-		
ZnSO <sub>4</sub> .7H <sub>2</sub> O - Zinc sulfate	0.88		
CuSO <sub>4</sub> .7H <sub>2</sub> O - Copper sulphate	0.2		
H <sub>3</sub> BO <sub>3</sub> - Boric acid	2.04		
Na2MoO4.2H2O - Ammonium molybdate	0.26		
Fe-EDTA - EDTa Iron	1000 mL		

Table 1. Quantitative of the fertilizers used in the preparation of mineral nutrient solutions.

Source: Furlani (1995).

Table 2. Chemical composition of mineral nutrient solutions.

Solution				
Mineral salts	Furlani			
g 1000 L <sup>-1</sup> of water				
NO3 - Nitrate	200.44			
NH4 – Ammonium	16.51432			
P - Phosphor	32.70			
K - Potassium	310.275			
Ca - Calcium	168			
Mg - Magnesium	24.65			
S - Sulfur	32.5			
Mn - Manganese	0.636714			
Zn - Zinc	0.199144			
Cu - Copper	0.0671			
Bo - Boron	0.356592			
Mo - Molybdenum	0.114452			
Fe - Iron	2.234			

Source: Furlani (1995).

The volume of the solution that fed the system (NFT) during lettuce cultivation was stored in plastic buckets with a capacity of 20 L, however, to avoid wasting solution by overflowing the bucket, the volume of 17 L. was used. In order to keep the nutrient solution within the recommended temperature limits for hydroponic cultivation, the buckets containing the solution were coated with 5mm thick Ethylene Vinyl Acetate (EVA) sheets, for the volume recorded after 24:00 h.

The quantification of the volume evapotranspirated by plants (VETc), depending on the nutrient solutions, was performed daily, completing the volume of the reservoir to the level of 17 liters, using a cylinder graduated in milliliters according to Equation 1.

VETc was considered as a quantitative variable to establish a polynomial regression analysis.

 $VETc = \frac{Vi}{n \Delta t}$ 

(1)

VETc - evapotranspirated volume, in L plant<sup>-1</sup> day<sup>-1</sup>;

Vi - volume of solution consumed within 24:00h;

 $\Delta T$  - time interval between readings, days;

n - number of plants in the profile in the time interval,  $\Delta T$ .

During the conduction of the experiment, the monitoring occurred daily, at intervals of 24:00h, taking readings of the solutions and the calibration of the hydrogen potential (pH), the electrical conductivity (CE), using portable equipment for the reading of pH, CE and total dissolved solids (TDS)/Temperature model HI 9811-5. After calibration, the pH of the solutions was adjusted, keeping them close to neutrality, using a solution of sodium hydroxide (NaOH) or Hydrochloric acid (HCL) (1mol L<sup>-1</sup>). The pH variation was made considering the average molar concentration of hydrogen ions (H +) using the expression pH = -log [H +].

The EC levels of all solutions were maintained taking into account 20% above or 20% below the initial EC. When the EC in each solution appeared 20% higher than the initial, the replacement was performed with rain water. When the EC presented 20% less than the initial, the replacement was made with the stock solution of each nutrient solution. The management of nutrient solutions was performed daily by replacing the water consumed, monitoring the EC and pH keeping it close to neutrality, using a solution of NaOH or HCL (1mol  $L^{-1}$ ) and regardless of treatments, the nutrient solutions were changed every seven days.

The sowing of curly lettuce (Cultivar 1 = Elba, Cultivar 2 = Cristina and Cultivar 3 = Veneranda) were done in trays and in phenolic foam of three cm in diameter and two cm in height, previously rinsed with running water in order to eliminate possible acidic compounds remaining from its manufacture. During the first six days, the foams were moistened only with water from the city of Campina Grande-PB; on the 7th, 13th and 19th days the solution S1 was added, so that it presented, respectively, 33.33; 66.66 and 100 % of the nutritional concentration suggested by Furlani et al. (1995). The cultivars were placed in the definitive hydroponic profile 24 days after germination.

The water used for the formulation of nutrient solutions came from the precipitation stored in a cistern and was analyzed for physical-chemical characteristics at the Irrigation and Salinity Laboratory (LIS/DEAg/UFCG) (Table 3).

Determinations	Rain water
pH	8.09
Electric conductivity (dS m <sup>-1</sup> )	0.156
Calcium (mmol <sub>c</sub> /L)	1.20
Magnesium (mmol <sub>c</sub> /L)	0.15
Sodium (mmol <sub>c</sub> /L)	0.12
Potassium (mmol <sub>c</sub> /L)	0.07
Chlorides (mmol <sub>c</sub> /L)	0.50
Carbonates (mmol <sub>c</sub> /L)	0.20
Bicarbonate (mmol <sub>c</sub> /L)	0.90
Sulfates (mg L <sup>-1</sup> )	Absent
Sodium adsorption ratio (SAR)	0.15
Class of water for irrigation	$C_1$

Table 3. Chemical composition of mineral nutritive solutions.

Source: Data from the present research.

Depending on the treatments, lettuce cultivars were evaluated 22 days after transplanting to the definitive hydroponic profile for the following parameters: total chlorophyll using the SPAD-50 portable meter, chlorophylls (*Chl a, Chl* and Chl total), carotenoids and Vitamin C.

Total chlorophyll was determined using the SPAD-502 portable chlorophyll meter (*Soil-Plant Analysis Development*) Section. Minolta Camer Co., Ltd, Japan.

The contents of Chl a, Chl b, total Chl, and carotenoids were determined by the method described by Lichtenthaler (1987), and the analyzes were quantified with UV-1000A Digital Spectrophotometer. The absorbance of the solution was obtained by spectrophotometry in the wavelengths of 470, 645 and 663 nm.

For the evaluation of Vitamin C, 15 g of whole sample and preparation in 250 ml Erlenmeyers containing 50 ml of 1% oxalic acid were used. Solutions used: I - 1% oxalic acid solution (1 g of oxalic acid PA was weighed and diluted in deionized water to 100 m1); II - Standard ascorbic acid solution 1 mg.m1<sup>-1</sup> (0.05 g of standard ascorbic acid was accurately weighed, stored away from light. Transfer to a 50 ml volumetric flask. Dilute to volume with oxalic acid solution 1%. The solution was prepared at the time of use) and; III-2.6 dichlorophenolindophenol standard solution (0.05 g) of dichloroindophenol was weighed, which was stored in a desiccator with soda and dissolved with 50 ml of deionized water in a 200 ml volumetric flask. It was shaken vigorously and when the dye dissolved, diluted to 200 ml of deionized water, filtered into an amber flask, stored in the dark and in the refrigerator.

The 15 g of whole sample was subjected to centrifugation in a blender immediately after collection, strained and transferred 2.0 ml of the standard ascorbic acid solution three times to different 250 ml Erlenmeyers flasks containing 50 ml of oxalic acid to 1%. It was titrated quickly with indophenol solution through a 50 ml burette, until a light, but distinct, persistent pink color. Each titration consumed between 15 to 17 ml of indophenol solution. Similarly, three blanks are titrated in the same way using deionized water instead of ascorbic acid solution. After decreasing the indophenol solution spent on the titration, the average of the determination of the blanks, the indophenol concentration was calculated as mg of ascorbic acid equivalent to 1.0 ml of reagent. The calculation and expression of the results was performed according to Brazil (1986).

The data obtained were submitted to analysis of variance by the F test at 1 and 5% probability. When there was a significant effect in the analysis of variance, the averages obtained in the subplots (cultivars) were compared using the Tukey test and for plots (solutions) regression was applied. The values of Chlorophyll A and Caratenoids did not follow the assumptions of the normality test (Shapiro-Wilk) to be submitted to ANOVA, so the means were compared by Kruskal and Wallis nonparametric statistics. Statistical analyzes were performed using the SISVAR statistical software (FERREIRA, 2014).

# 3. Results and Discussion

Analyzing Table 4, only the cultivar factor significantly influenced the total chlorophyll content analyzed by the indirect SPAD index at the level of 1% probability (p <0.01) in cultivars (C).

Table 4 - Analysis of variance of the total chlorophyll contents in the leaves by the SPAD index evaluated in the greenhouse at the end of the hydroponic cultivation of the three cultivars of curly lettuce submitted to different treatments.

FV	GL	QM
		SPAD
Solution (S)	6	0.09 <sup>ns</sup>
Block	2	$0.07^{ns}$
Residue 1	11	0.05
Cultivar (C)	2	0.63**
C * S	12	$0.06^{ns}$
Residue 2	92	0.03
CV 1		7.39
CV 2		6.75
Overall average		2.95

DF - degree of freedom; SV- source of variation; MS - medium square; ns not significant; \*\* significant at 1% probability (p <0.01); by the F test; CV = coefficient of variance. Plots and subplots, respectively.

Source: Data from the present research.

The levels of total chlorophyll in lettuce leaves by the SPAD index can be seen in Figure 2. The highest value for total chlorophyll was obtained in the cultivar Veneranda whose average corresponded to 3.08 by the SPAD index.

**Figure 2** - Indirect determination of total chlorophyll levels in the leaves by the SPAD index as a function of the isolated effect of the lettuce cultivars Elba, Cristina and Veneranda at the end of the experiment.



Source: Data from the present research.

Santos et al. (2010) evaluated the chlorophyll content in lettuce grown in hydroponic, conventional and organic systems; and obtained the following values of 13.16; 12.72 and 13.22 respectively, these values are also different from those found in this research. Possibly the chlorophyll index found in this study was affected by the levels of sodium chloride (NaCl) added in the nutrient solutions. These fluctuations in results show the importance of further research on the interference of salinity on different plant tissues. Analyzing the data in Figure 2, with those of Rosa et al. (2014) who studied the culture of green and purple mimosa lettuce in a hydroponic cultivation system with electrical conductivity of the solution between 1.85 and 2.00 dS/m, the authors found that green mimosa lettuce had lower chlorophyll contents.

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Figure 3 shows contents of *Chl a* as a function of the nutritional solutions  $S_1 = 1.9$ ;  $S_2 = 2.4$ ;  $S_3 = 2.9$ ;  $S_4 = 3.4$ ;  $S_5 = 3.9$ ;  $S_6 = 4.4$  and  $S_7 = 4.9$  dS m<sup>-1</sup> and from the cultivars Elba, Cristina and Veneranda at the end of the experiment. The highest averages were found in the  $C_1S_2$  treatments (Elba/ $S_2 = 2.4$  dS m<sup>-1</sup>) and  $C_1S_3$  (Elba/ $S_3 = 3.9$  dS m<sup>-1</sup>), with the values of 57 and 54 mg.g<sup>-1</sup> in the fresh leaf mass (MF). Comparing the levels of chlorophyll found in this research with those of Cardoso et al.

(2018) who worked with cultivars of the commercial groups Lisa Repolhuda and Crespa Solta and observed that the Crespa Repolhuda group had levels of Chl a with an average of  $6.58 \text{ mg/g}^{-1}$ , this result is lower than this research.

**Figure 3** - Determination of chlorophyll contents (Chl to mg.g<sup>-1</sup> MF) of lettuce cultivars Elba, Cristina and Veneranda as a function of nutrient solutions  $S_1 = 1.9$ ;  $S_2 = 2.4$ ;  $S_3 = 2.9$ ;  $S_4 = 3.4$ ;  $S_5 = 3.9$ ;  $S_6 = 4.4$  and  $S_7 = 4.9$  dS m<sup>-1</sup> at the end of the experiment. Averages followed by the same letter do not differ.



Source: Data from the present research.

It is observed in Table 5 that there was no significant effect for the levels of *chl* b and total *chl* in the factor nutrient solution (S). As for the results of the isolated effect on the cultivar factor (C), the levels *chl* b and *total chl* showed a significant effect at the level of 1% probability (p < 0.01). For the results of the interaction of the nutrient solution within cultivars and vice versa, it was observed that there was no significant effect on the levels *chl* b and total *chl* (Table 5).

FV	GL		QM	
		Cha b	Cha total	
Solution (S)	6	0.54 <sup>ns</sup>	0.76 <sup>ns</sup>	
Block	2	0.25 <sup>ns</sup>	0.46 <sup>ns</sup>	
Residue 1	11	0.30	0.25	
Cultivar (C)	2	2.36**	$2.29^{**}$	
C * S	12	0.27 <sup>ns</sup>	0.34 <sup>ns</sup>	
Residue 2	92	0.30	0.27	
CV 1		17.07	13.79	
CV 2		17.2	14.39	
Overall average		3.21	3.60	

**Table 5** - Analysis of variance of the levels of chlorophyll b and total chlorophyll in the fresh weight (MF) of the three lettuce

 cultivars submitted to different treatments with saline solutions.

DF - degree of freedom; SV- source of variation; MS - medium square; ns not significant; \*\* significant at 1% probability (p < 0.01); by the F test; CV = coefficient of variance. Plots and subplots, respectively. Source: Data from the present research.

Regarding *Chl b* levels, to meet the statistical assumptions of normality, the data were transformed into  $\ln (x)$ , thus the behavior observed in Figure 4, the content of *Chl b* contents according to the cultivars Elba, Cristina and Veneranda at the end of the experiment, the same figure shows that the highest mean for *Chl b* was found in the cultivar Veneranda, whose unprocessed value was (35.97 mg.g<sup>-1</sup> MF).

**Figure 4** - Determination of the levels of chlorophyll b (*Chl* b mg.g<sup>-1</sup> MF) as a function of the isolated effect of cultivars Elba, Cristina and Veneranda at the end of the experiment. Averages followed by the same letter do not differ.



Source: Data from the present research.

Comparing these results with those of Xavier et al. (2019), who working under conditions similar to this research obtained for the content of *Chl b* in the cultivar Thais were (17.54 mg/g<sup>-1</sup> MF), this value is lower than in this research. Probably this lower value of *Chl b* found by the authors was due to the use of a nutrient solution prepared with waste water from the UASB (*Upflow Anaerobic Sludge Blanket*) reactor that possibly the amount of macro and micronutrients in this water did not favor the development of the plants. Cardoso et al. (2018) analyzed cultivars from the commercial groups Lisa Repolhuda and Crespa Solta and observed that the group Crespa Repolhuda presented *Chl b* contents with an average of 5.86

mg/g<sup>-1</sup>, these results are different from those of this research.

In order to meet the statistical deductions of normality, the data for total Chl were transformed into ln (x), thus it can be seen in Figure 5 that the highest mean of total Chl content was in the cultivar Veneranda ( $52.06 \text{ mg/g}^{-1}$  of MF). The cultivars Elba and Cristina had the following averages of total Chl:35.04 and  $27.07 \text{ mg/g}^{-1}$  of MF, respectively and when compared at the end of the experiment, they did not differ statistically. The total Chl values found in this research were lower than those found by Xavier et al. (2019), who verified a total Chl content of  $242 \text{ mg/g}^{-1}$  MF to Vanda curly lettuce using the solution with domestic wastewater (raw sewage).

**Figure 5** - Determination of chlorophyll contents (Chl total  $mg.g^{-1}$  MF) as a function of the isolated effect of cultivars Elba, Cristina and Veneranda at the end of the experiment. Averages followed by the same letter do not differ.



Source: Data from the present research.

Figure 6 shows the content of carotenoids in cultivars Elba, Cristina and Veneranda as a function of nutrient solutions  $S_1 = 1.9$ ;  $S_2 = 2.4$ ;  $S_3 = 2.9$ ;  $S_4 = 3.4$ ;  $S_5 = 3.9$ ;  $S_6 = 4.4$  and  $S_7 = 4.9$  dS m<sup>-1</sup> at the end of the experiment. It appears that the highest mean was found in the C<sub>1</sub>S<sub>3</sub> treatment (Elba/S<sub>3</sub> = 3.4 dS m<sup>-1</sup>) whose value was 57 mg.g<sup>-1</sup> MFF. Moura et al. (2016) working with purple lettuce, fertilizer, without pesticides and with an organic compost produced from manure and crop residues from the garden in the development period of 20, 30 and 40 days after transplanting (DAT) found a high content of carotenoids (128.098 mg.g<sup>-1</sup>) at 40 DAT in plants. Silva et al. (2016) found that there was an increase in the levels of carotenoids as there was an increase in the application of water to plants.

**Figure 6** - Determination of the levels of carotenoids (mg.g<sup>-1</sup> MF) in the leaves of the cultivars of curly lettuce according to the nutrient solutions  $S_1 = 1.9$ ;  $S_2 = 2.4$ ;  $S_3 = 2.9$ ;  $S_4 = 3.4$ ;  $S_5 = 3.9$ ;  $S_6 = 4.4$  and  $S_7 = 4.9$  dS m<sup>-1</sup> and cultivars C1 (Elba), C2 (Cristina) and C3 (Veneranda) at the end of the experiment. Averages followed by the same letter do not differ.



Source: Data from the present research.

Most research on plant ripening is based on assessments of color, total soluble solids and acidity (Motta et al., 2015), which undergo considerable changes during development. These basic attributes are essential to establish the stage of maturation, and important in the sensory detection of product quality by consumers (Carvalho et al., 2011; Ferreira et al., 2012). Another important attribute for the analysis of the quality of lettuce are carotenoids, which are substances that provide several benefits for human health.

Table 6 shows that the vitamin C content (CVT) in the cultivar factor (C) had a significant effect at the level of 1% probability (p <0.01). As for the results of the isolated effect of the nutrient solution (S), it is observed that the CVT had a significant effect at the level of 5% probability ( $0.01 \le p < 0.05$ ). For the results of the interaction of the nutrient solution within cultivars and vice versa, it is observed that there was no significant effect on the CVT (Table 6).

FV	GL	QM
		Vitamina C-TVC mg.100g <sup>-1</sup>
Solution (S)	6	9.42*
Block	2	11.70
Residue 1	11	4.49
Cultivar (C)	2	121.23**
C*S	12	8.68 <sup>ns</sup>
Residue 2	92	4.15
CV 1		3.92
CV 2		3.76
Overall average		54.15

**Table 6** - Analysis of variance of vitamin C levels (CVT) in the fresh mass (MF) of the three lettuce cultivars submitted to different treatments with saline solutions.

DF - degree of freedom; SV- source of variation; MS - medium square; ns not significant; \*\* significant at 1% probability (p < 0.01); by the F test; CV = coefficient of variance. Plots and subplots, respectively. Source: Data from the present research.

It can be seen in Figure 7A that the nutrient solutions showed a linear behavior regarding the CVT in lettuce plants for all the electrical conductivities tested; and there was no difference statically between them. When evaluating the cultivars in Figure 7B, it appears that the cultivars Cristina and Veneranda have the highest levels of vitamin C, whose averages were 54.80 and 56.17 mg.100g<sup>-1</sup>, respectively.

**Figure 7** - Determination of vitamin C content (CVT mg.100g<sup>-1</sup>) of curly lettuce as a function of the isolated effect of S1 nutrient solutions; S<sub>2</sub>; S<sub>3</sub>; S<sub>4</sub>; S<sub>5</sub>; S<sub>6</sub> and S<sub>7</sub> (A) and cultivars Elba, Cristina and Veneranda (B). S<sub>1</sub> = 100% of the Furlani solution prepared with rain water and (S<sub>2</sub>; S<sub>3</sub>; S<sub>4</sub>; S<sub>5</sub>; S<sub>6</sub> and S<sub>7</sub>) also prepared using rain water with sodium chloride (NaCl) being added in order to have the following electrical conductivities: S<sub>2</sub> = 2.4; S<sub>3</sub> = 2.9; S<sub>4</sub> = 3.4; S<sub>5</sub> = 3.9; S<sub>6</sub> = 4.4 and S<sub>7</sub> = 4.9 dS m<sup>-1</sup>.





It can be seen in Figure 7A that the nutrient solutions showed a linear behavior regarding the CVT in lettuce plants for all electrical conductivities tested; and there was no difference statically between them. When evaluating the cultivars in Figure

7B, it appears that the cultivars Cristina and Veneranda have the highest levels of vitamin C, whose averages were 54.80 and  $56.17 \text{ mg}.100 \text{ g}^{-1}$ , respectively.

Comparing the results found in this research, it was found that the CVT were superior to those found by Ohse et al. (2001), where they found in their research a variation in the levels of vitamin C from 41.89 to 19.24 mg.100 g<sup>-1</sup>. Correlating the results of the levels of vitamin C found in this research with those recommended by the Brazilian Food Composition Table of 2011 (TBCA) it was found that the CVT found in the present study are higher than those of TBCA, where it reports that in every 100 g of fresh lettuce contains 15.6 mg of vitamin C.

Comparing the results of the levels of vitamin C (mg.100 g<sup>-1</sup>) found in this research, with those of Xavier et al. (2019), where they worked under similar conditions, with lettuce cultivars and nutrient solutions prepared with waste water and saline water, the authors obtained levels of vitamin C (57.33 mg.100 g<sup>-1</sup>) for the cultivar Vanda, this result corroborates those found in this research. Gonçalves & Coringa (2017) evaluated the vitamin C content of lettuce collected in the local commerce of Cuiabá/MT grown in a hydroponic system and found levels of vitamin C of 28.16 mg.100 g<sup>-1</sup>. Polette et al. (2018), found vitamin C values for curly lettuce (SVR-2005) and Vanda (TE-112) of 6.1 mg.100 g<sup>-1</sup> and 6.5 mg.100 g<sup>-1</sup> respectively, values that are much lower than found in this research.

Sarmento et al. (2014) studied the vitamin C content of lettuce and observed the increase in response to the increase in the salinity of the nutrient solution, presenting an ascorbic acid content of 24.54 mg 100g-1 when irrigated with a nutrient solution with 1.1 dS EC m<sup>-1</sup> and maximum value of 26.67 mg.100g<sup>-1</sup> in nutrient solution with EC of 5.7 dS m<sup>-1</sup>, these are lower than those found in this research.

# 4. Conclusion

The nutritional solutions showed linear behavior for all treatments and did not differ statistically from each other.

Chlorophyll (Chl a) obtained the highest levels in treatments  $C_1S_2$  (Elba/ $S_2 = 2.4$  dS m<sup>-1</sup>) and  $C_1S_3$  (Elba/ $S_3 = 3.9$  dS m<sup>-1</sup>).

The cultivar Veneranda had the highest levels for Chlorophyll (Chl b) and (Chl total).

The plants of the C1S3 treatment (Elba /  $S_3 = 3.4$  dS m-1) had the highest carotenoid content.

The highest levels of vitamin C were found in the cultivars Cristina and Veneranda.

The highest value for total chlorophyll was obtained for the cultivar Veneranda.

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